

5 metabolite production has been isolated. Preferred heterologous cells include, but are not limited to, *S. cerevisiae*, *E. coli*, *A. nidulans*, and *Candida sp.*, and *N. crassa*. Particularly preferred are fungal heterologous cells. In an embodiment of the third aspect, the method
10 comprises: (a) selecting a nucleic acid comprising a polynucleotide encoding a protein regulator of secondary metabolite production; (b) mutating the nucleic acid to create a plurality of nucleic acid molecules encoding variant regulator proteins of secondary metabolite
15 production; and (c) selecting a mutagenized nucleic acid encoding a variant regulator protein with increased activity in a homologous cell than the cognate, wild-type protein.

As used herein, the phrase "homologous cell" refers
20 to a system for gene expression, i.e., an organism for gene expression, that is the organism from which the regulator protein of secondary metabolite production has been isolated. Preferred homologous cells are fungal homologous cells, including, but not limited to,
25 *Aspergillus sp.*, *Penicillium sp.*, *Acremonium chrysogenum*, *Yarrowia lipolytica*, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus sp.*, *Claviceps sp.*, *Trichoderma sp.*, *Tolypocladium sp.*, *Tricotheicum sp.*, *Fusidium sp.*, *Emericellopsis sp.*, *Cephalosporium sp.*, *Cochliobolus sp.*,
30 *Helminthosporium sp.*, *Agaricus brunescens*, *Ustilago maydis*, *Neurospora sp.*, *Pestalotiopsis sp* and *Phaffia rhodozyma*. (See, Fungal Physiology, Chapter 9 (Secondary(Special) Metabolism), Griffin, D. H., John Wiley & Sons, Inc.; ISBN: 0471166154).

35 In certain embodiments of the third aspect, the method further comprises selecting a variant regulator protein that also increases production of a secondary metabolite in a cell when compared to the cognate, wild-type protein. In certain embodiments thereof, the cell is
40 a fungal cell. In certain embodiments thereof, the cell is a heterologous cell, preferably selected from the group

5 consisting of *S. cerevisiae*, *E. coli*, *A. nidulans*, *Candida*
sp., and *N. crassa*.

In certain embodiments thereof, the cell is a homologous cell, preferably selected from the group consisting of *Aspergillus* sp., *Penicillium* sp., *Acremonium* 10 *chrysogenum*, *Yarrowia lipolytica*, *Nodulisporium* sp., *Fusarium* sp., *Monascus* sp., *Claviceps* sp., *Trichoderma* sp., *Tolypocladium* sp., *Tricotheicum* sp., *Fusidium* sp., *Emericellopsis* sp., *Cephalosporium* sp., *Cochliobolus* sp., *Helminthosporium* sp., *Agaricus brunescens*, *Ustilago* 15 *maydis*, *Neurospora* sp., *Pestalotiopsis* sp., and *Phaffia rhodozyma*.

Certain embodiments of the aspects of the invention relate to regulator proteins that promote secondary metabolite production by increasing transcription of one 20 or more genes involved with secondary metabolite production. These wild-type sequences may be selected for mutagenesis to create a plurality of variant regulator proteins. The activity of these transcription-activating variant regulator proteins may be determined by measuring 25 the activity of a reporter gene having the appropriate promoter sequences. These tests are done in a homologous and/or a heterologous cell. Certain embodiments of aspects of the invention are directed to fungal regulator proteins with transcription-activating activity that is 30 tested in fungal heterologous and homologous cells.

Reporter genes are useful for isolating transformants expressing improved variant regulator proteins. The reporter genes may be operably linked to a promoter sequence that is normally regulated by the wild-type 35 regulator protein. Reporter genes include, but are not limited to, genes encoding β -galactosidase (*lacZ*), β -glucuronidase (*GUS*), β -glucosidase, amylase and invertase, amino acid biosynthetic genes, e.g., the yeast *LEU2*, *HIS3*, *LYS2*, *TRP1* genes (or homologous genes from other fungi, 40 such as filamentous fungi, that encode proteins with the similar functional activities), nucleic acid biosynthetic

5 genes, e.g., the yeast *URA3* and *ADE2* genes (or homologous genes from other fungi, such as filamentous fungi, that encode proteins with the similar functional activities), the mammalian chloramphenicol transacetylase (CAT) gene, or any surface antigen gene for which specific antibodies
10 are available. A reporter gene can also be a neomycin phosphotransferase(neo) gene, which encodes neomycin, kanamycin resistance gene and G418 (geneticin) resistance gene. A reporter gene may encode a protein detectable by luminescence or fluorescence, such as green fluorescent
15 protein (GFP). Reporter genes may additionally or alternatively encode any protein that provides a phenotypic marker, for example, a protein that is necessary for cell growth or viability, or a toxic protein that causes cell death. Alternatively, the reporter gene
20 may encode a protein detectable by a color assay leading to the presence or absence of color.

The choice of reporter gene will depend on the type of cell to be transformed. Preferred reporter genes are those that are operable in fungal cells. It is preferable
25 to have two reporter genes within the cell. One reporter gene, when expressed, provides a growth advantage to transformed cells that are expressing the variant regulator protein. This allows for the isolation of such transformants though selective pressures. The other
30 reporter gene provides a colorimetric marker, such as the *lacZ* gene and its encoded protein, β -galactosidase. Alternatively, the second reporter provides a fluorescent or luminescent marker, such as green fluorescent protein (GFP).

35 In a fourth aspect, the invention provides a method of increasing production of a secondary metabolite comprising: (a) selecting a nucleic acid comprising a polynucleotide encoding a protein regulator of secondary metabolite production; (b) mutating the nucleic acid to
40 create a plurality of nucleic acid molecules encoding variant regulator proteins of secondary metabolite production; (c) selecting a variant regulator protein with